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Review

Biological effects of organic arsenic compounds in seafood†

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This review describes the results of our recent experiments concerning the *in vitro* biological effects of water-soluble organic arsenic compounds contained in seafood in murine immune effector cells using synthetic pure materials. A dimethyl organic arsenic compound in seaweed, viz. an arsenosugar, was weakly cytotoxic in murine alveolar macrophages during a 72 h incubation (50% lethal concentration *in vitro*, LC_{50} = 8 mmol dm^{-3}); conversely, it increased the cell viability of peritoneal macrophages at an optimal dose of 5 mmol dm^{-3}. Trimethyl arsenic compounds in marine animals, arsenocholine and arsenobetaine, were less toxic in murine splenocytes, thymocytes, Peyer's patch lymphocytes, peritoneal macrophages and alveolar macrophages *in vitro*, even over 10 mmol dm^{-3}. Interestingly, they significantly increased the cell viability of immature bone marrow cells at doses over 100 μmol dm^{-3}, and induced the maturation of bone marrow cells especially into granulocytes. The tetramethyl arsenic compound, tetramethylarsonium hydroxide, isolated from some lower marine animals had no *in vitro* cytolethality on murine immune effector cells. Taken together, organic arsenic compounds in seafood are not very toxic in living systems. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; organic arsenic; seafood; immunotoxicity; immunopharmacology; arsenosugar; arsenocholine; arsenobetaine; trimethylarsenic; tetramethylarsonium

INTRODUCTION

When arsenic is mentioned, most people often think of it as a lethal poison, and the adverse effects from exposure to this metalloid are considered among the top priority hazards in many countries.[1] Arsenic is a common constituent of the Earth's crust in its inorganic form, trivalent (arsenite) or pentavalent (arsenate), and it is widely distributed in soil and water.[2] Humans may encounter inorganic arsenicals in drinking water from wells drilled into arsenic-rich strata. It has been reported that arsenic poisoning has occurred in some countries, especially in Asia[3,4] and South America,[5,6] through the consumption of contaminated well water. The acute toxicity of inorganic arsenicals is very high; the LD_{50} (50% lethal dose *in vivo*) of arsenite in mice is 35 mg kg^{-1}.[7] Also, for more than a century, various carcinogenic effects of inorganic arsenicals on humans have been documented, mainly involving the skin and lung,[8] and recent epidemiological studies have indicated that the ingestion of inorganic arsenicals is related to cancer induction in the liver, kidney, urinary bladder and other internal organs.[9,10]

It has been well known that marine organisms, which are daily ingested as seafood in many countries, contain very high concentrations of arsenic, e.g. seaweed [the average arsenic concentration is about 30 μg g^{-1} (dry weight)], snails (78 μg g^{-1}), clams (4 μg g^{-1}), sea slugs (8 μg g^{-1}), sea urchins (23 μg g^{-1}), cuttlefish (4 μg g^{-1}), crustacea (30 μg g^{-1}) and fish (4 μg g^{-1}).[11] The limit for arsenic in drinking water in Japan, 10 μg dm^{-3}, is largely based on inorganic arsenicals; if this limit were applied to seafood, as 10 ng g^{-1}, most of the seafood would be deemed unfit for consumption, given that their contents are often 1000 times this concentration.[12] This

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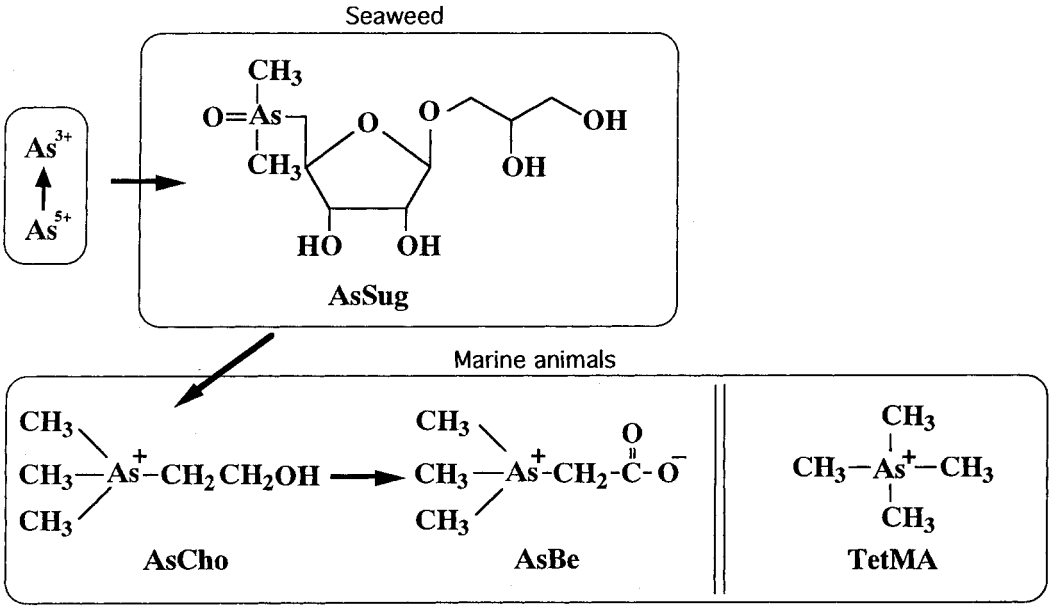

Figure 1. Organic arsenic compounds in marine organisms.

finding has caused great concern with respect to the health of people who often ingest considerable amounts of seafood. From 1977 to 1988, Edmonds and coworkers,[13–15] Norin and Christakopoulos[16] and Shiomi and coworkers[17–19] found that the arsenicals in seafood are generally not inorganic chemicals, but are in the form of water-soluble organic arsenic compounds. In the marine ecosystem, it has been demonstrated that trace amounts of inorganic arsenicals in sea water are probably taken up into seaweed, including Phaeophyceae, Rhodophyceae and Chlorophyceae, and are accumulated and metabolically methylated to dimethyl arsenic-containing riboses,[14,15] and (*R*)-(2′,3′-dihydroxypropyl) 5-deoxy-5-dimethylarsinoyl-*β*-D-riboside, namely arsenosugar (AsSug; see Fig. 1), was identified as a major dimethyl arsenic-ribose in seaweed by Edmonds and Francesconi.[15] These dimethyl arsenic compounds in seaweed are further methylated and converted into trimethyl arsenic compounds in many species of marine animals, including clams, snails, crab, lobster, shrimp and fish, and Edmonds *et al.*[13] also isolated trimethyl (carboxymethyl) arsonium zwitterion, namely arsenobetaine (AsBe; see Fig. 1), as a major trimethyl arsenic compound in marine animals. It has been suggested that AsBe is the final metabolite in the arsenic cycle of marine ecosystems because it is widely distributed in various species of marine animals. Norin and Christakopoulos[16] and Shiomi *et al.*[17] subsequently identified another water-soluble trimethyl arsenic compound, namely arsenocholine (AsCho), at low levels from certain kinds of shrimp and conch. The chemical structure of this new trimethyl arsenic compound was the trimethyl (2-hydroxyethyl)-arsonium cation (see Fig. 1),[16,17] and it was thought to be a precursor of AsBe.[20,21] On the other hand, Shiomi and coworkers also isolated a minor water-soluble tetramethyl arsenic compound from the branchia of a clam[18] and the skin of lower marine animals, such as the sea hare and the sea anemone,[19] and identified its chemical structure as the tetramethylarsonium salt (TetMA; see Fig. 1).[18,19] TetMA may have originated in the marine sediments and was probably trapped in the external tissues of these marine animals, because this tetramethyl arsenic compound was detected in only a very few marine animals and/or marine sediments.[22,23] Interestingly, a recent study indicated that TetMA was formed in roasted seafood, although it was not detected at all before cooking.[24] As described above, in many countries, people are daily ingesting considerable amounts of arsenicals through the consumption of seafood; thus, it is necessary to investigate the effects of these organic arsenic compounds, including AsSug, AsCho, AsBe and TetMA, on living systems. However, there have been relatively few reports about them because sufficient amounts of the pure compounds for biological experiments have not been obtained. Recently, we examined their biological effects in mammalian cells, especialy in immune effector cells, using pure synthetic materials.[25–28] This review describes the results of our recent experiments, which showed that these organic arsenic compounds may not be very toxic in living systems.

ARSENOSUGAR (AsSug)

Some kinds of Phaeophyceae and Rhodophyceae, such as *Hizikia fusiforme, Laminaria japonica, Ecklonia cava* and *Porphyra*

tenera, which are often ingested as seafood in Japan, contain very high concentrations of AsSug, about 12–47 μg g^{-1} (dry weight),[11] and the average daily consumption of these brown and red algae by the Japanese is reported to be about 2–3 g with a calculated high of 12 g dry weight.[29] Le *et al.*[12] demonstrated that AsSug in brown kelp remained for more than 3 days in the human body after oral ingestion. However, little is known about the effect of AsSug on living systems. In 1997, we first reported the *in vitro* biological effects of AsSug on mammmalian cells, murine peritoneal macrophages and alveolar macrophages, using synthetic AsSug.[26] AsSug was synthesized from 1-*O*-acetyl-tri-*O*-benzoil-*β*-D-ribofuranose, (*S*)-1,2-*O*-isopropyl-idene glycerol and dimethylarsinous iodide by a modified method of McAdam and Stick.[30] As a result, AsSug had no cytotoxicity in both types of macrophage at the μmol dm^{-3} level; however, it induced different and interesting cellular responses in both types of macrophage at the high concentrations of 1–10 mmol dm^{-3}. AsSug enhanced the viability of peritoneal macrophages (by about a 1.6-fold increase compared with the control cells which were incubated with medium alone) at an optimal dose of 5 mmol dm^{-3} within a 48 h incubation time; conversely, it showed weak cytotoxicity and induced apoptosis-like cell death toward alveolar macrophages (50% lethal concentration *in vitro* LC_{50} = 8 mmol dm^{-3}). It is possible that the different effects of AsSug on peritoneal macrophages and alveolar macrophages may be due to the difference in the characteristics of these two local macrophages, such as redox functions. In our preliminary experiment, the *in vivo* acute toxicity of AsSug was found to be very weak (LD_{50} was >6 g kg^{-1} in mice when administered orally; unpublished data). Considering these facts, it is suggested that one-time consumption of AsSug contained in seaweed is not very toxic to the health of people who often consume seaweed as food; however, Le *et al.*[12] reported that a part of AsSug in brown kelp was converted to toxic dimethylarsinic acid in the human body after oral ingestion. Therefore, further *in vivo* examinations are needed to clarify the distribution, metabolism, excretion and chronic toxicity of AsSug. Also, the evaluation of the pharmacological effects of AsSug and/or other arsenic-containing riboses is of interest because the chemical structure of AsSug is unusual and interesting.

ARSENOCHOLINE (AsCho)

AsCho was detected at low levels, about 0.3% of total arsenicals, from shrimp[16] and conch,[17] and it is thought to be a possible precursor candidate of AsBe in the marine food chain.[20,21] In 1992, Kaise *et al.*[31] demonstrated the effects of AsCho on living systems using synthetic pure material, and found that AsCho had a weak but significant acute toxicity in murine models. The LD_{50} values of AsCho in mice were 187 mg kg^{-1} and 6.54 g kg^{-1} when administered intravenously and orally respectively.[31] This report suggested that AsCho was slightly but significantly toxic in mammals; however, there was no more data about the toxicity of AsCho. In 1996, we subsequently reported the *in vitro* cytotoxicity of AsCho in murine immune effector cells using synthetic AsCho.[25] AsCho was synthesized from trimethylarsine that reacted with 2-bromo-ethanol.[32] As a result, AsCho was less toxic even at a concentration over 10 mmol dm^{-3} in murine peritoneal macrophages, alveolar macrophages and splenocytes *in vitro*. Also, we recently showed that AsCho was not cytolethal in murine thymocytes, Peyer's patch lymphocytes and bone marrow cells *in vitro* even over 10 mmol dm^{-3}.[33] These findings suggest that AsCho is not very toxic in living systems. Interestingly, it slightly augmented the viability of bone marrow cells (about a 1.3-fold increase in the viability of cells compared with that of control cells during a 72 h incubation) at high concentrations over 100 μmol dm^{-3}.[33] As described below, this unique biological action was also found to be more effective with AsBe, but was not observed for any other arsenic compounds.[28] Marafante *et al.*[34] and Kaise *et al.*[31] examined the metabolism of AsCho and reported that it was converted to AsBe and rapidly excreted into the urine after oral administration; however, we found that AsCho was not converted into any other arsenicals, including AsBe, in murine bone marrow cells *in vitro*.[33] It is likely that the increasing effect of AsCho on the viability of bone marrow cells is due to the chemical structure of AsCho.

ARSENOBETAINE (AsBe)

It is well known that AsBe is contained at high levels in various kinds of marine animals that are ingested daily as seafood in many countries; most of the arsenic containined in marine animals is AsBe. In 1985, Kaise *et al.*[7] first reported on the acute toxicity of AsBe using synthetic pure AsBe and found that it had no acute toxicity in murine models even over 10 g kg^{-1} when it was administered orally. Subsequently, using this synthetic material, we observed that the *in vitro* cytotoxicity of AsBe was very weak compared with that of inorganic arsenicals in cultured murine peritoneal macrophages, alveolar macrophages and splenocytes; it was less toxic even at a concentration over 10 mmol dm^{-3}.[25] AsBe was synthesized from trimethyl-arsine that reacted with ethyl *β*-bromo-propionate in an atmosphere of carbon dioxide.[13] Additionally, Oya-Ohta *et al.*[35] reported that AsBe did not induce chromosomal aberrations in human fibroblasts, and Irvin and Irgolic[36] also documented that AsBe had no embryotoxicity using rat models. Taken together, we believe that AsBe has no biological effects, including toxic effects, in living systems; however, we recently demonstrated that AsBe interestingly modulated the cell viability of immature bone marrow cells *in vitro*, although it had no biological effects at all in lymphocytes, such as thymocytes and Peyer's patch lymphocytes.[28] AsBe significantly enhanced the viability of bone marrow cells in a dose-

dependent manner during a 72 h incubation; an approximate twofold increase in the viability of cells compared with that of control cells cultured with the medium alone was observed using a μmol dm^{-3} level of AsBe. In morphological investigations, AsBe enhanced the number of large mature adherent cells, especially granulocytes, during a 72 h bone marrow culture. However, AsBe did not cause proliferation of bone marrow cells at all, as determined by a colony-forming assay using a gelatinous medium. The reasons why AsBe enhances the survival of immature bone marrow cells are not yet precisely known. AsBe might first enhance the cell adhesion ability of bone marrow cells during the initial 24 h incubation and continuously increase the survival of these cells, resulting in inducing the maturation of these surviving cells into large adherent cells, especially granulocytes, during the 72 h incubation. It is well known that the oxidation state of the arsenic molecule influences the type and severity of the biological effects.[12,37] For example, arsenic has a very high affinity for thiol groups when it has a trivalent oxidation state; in contrast, it can replace phosphate when it has a pentavalent oxidation state. It is suggested that the initial enhanced adhesion ability of bone marrow cells induced by AsBe may depend on the conformational changes in the cell surface proteins by the binding of AsBe on the cell surface thiol groups and/or phosphate. It has also been reported that the biological effects of arsenic compounds depend on their chemical structures.[12,37] In one study, we showed that significant modulating effects on the viability of bone marrow cells were observed only with AsBe, and not with any other inorganic and organic arsenic compounds, such as sodium arsenite and trimethylarsine oxide.[28] Glycinebetaine, the nitrogenous analogue of AsBe, did not show any potent effect on bone marrow cells, and the simultaneous addition of trimethylarsine oxide and glycinebetaine also did not influence them. Furthermore, we demonstrated that AsBe was not methylated or demethylated in bone marrow cells with a fully automated continuous arsine-generation system using gas chromatography–mass spectrometry. Taken together, these findings suggest that the chemical structure of AsBe is a very important factor, at least in part, for the expression of the significant effects on the viability of immature bone marrow cells. As described above, the AsBe-induced weak modulating effect on the survival of bone marrow cells was observed with AsCho, which has a chemical structure similar to that of AsBe.[33] Additionally, this biological effect was not observed with any other methyl arsenic compounds, such as monomethylarsonic acid, dimethylarsinic acid[38] and tetramethylarsonium hydroxide.[27]

It is very interesting that this unique biological effect was found with AsBe, a major arsenic compound contained in large quantities in the various marine animals ingested daily as seafood in many countries. In immunocompromised hosts, such as individuals receiving drug therapy or irradiation and patients with acquired immunodeficiency syndrome, severe infectious diseases are frequently caused because the number of leukocytes, including granulocytes and macrophages (which are essential immunological components for the initial response to infectious microorganisms as phagocytes), are decreased. Therefore, it is likely that AsBe has a possible application as a biological response modifier to increase the number of granulocytes and macrophages by increasing the cell survival of immature bone marrow cells without fatal toxic side effects. Additionally, in that study, there were significant additive-like increasing effects on the number of granulocytes and macrophages that originated from bone marrow cells between AsBe and a low dose (1 U ml^{-1}) of recombinant murine granulocyte/macrophage colony-stimulating factor (rMu GM-CSF), and significant additive-like increasing effects were observed on the number of both granulocytes and macrophages originated from bone marrow cells.[28] GM-CSF is one of the promising cytokines for use as a biological response modifier, but it also has severe inflammatory toxic side effects when it is used at high doses; thus, the combination of AsBe and a low dose of GM-CSF may be useful for the clinical application of these reagents. However, some researchers have reported that AsBe ingested by consuming seafood was rapidly excreted, within 36 h, into the urine unchanged by the human subjects.[12,39,40] Kaise *et al.*[7] also previously described that AsBe was detected in the urine in the non-metabolized form after an oral administration using synthetic AsBe in murine models. Thus, it is necessary to investigate the *in vivo* effect of AsBe in bone marrow cells, including detailed examinations for drug design and administration routes. We are now examining the *in vivo* effects of AsBe on immune systems using mice models, and this work will be published in the near future.

TETRAMETHYLARSONIUM SALT (TetMA)

In 1987, Shiomi and coworkers detected a new minor water-soluble organic arsenic compound, TetMA, from the branchia of a clam, *Meretrix lusoria*,[18] and some lower marine animals, such as the sea hare, *Aplysia kurodai*, and the sea anemone, *Parasicyonis actinostoloides*.[19] The chemical structure of TetMA caused great concern with respect to the health of people because the tetramethylammonium ion (namely tetramine), the nitrogenous analogue of the tetramethylarsonium ion, has been known to be a causative compound of numerous intoxications in Japan due to the ingestion of sea snails, such as *Neptunea arthritica*.[41,42] Shiomi *et al.*[41] and Kaise and Fukui[43] examined the lethal toxicity of the tetramethylarsonium ion in mice using synthetic tetramethylarsonium iodide or chloride, and found that these halide tetramethylarsonium salts showed significant acute toxicity; their LD_{50} values were 890 mg kg^{-1} or 580 mg kg^{-1} respectively. We also demonstrated that tetramethyl-arsonium iodide exhibited a weak *in vitro* cytotoxicity in cultured

murine splenocytes; its LC_{50} was 6 mmol dm^{-3}.[25] Taken together, it is believed that the tetramethylarsonim ion has a weak but significant toxicity in mammalian living systems; however, other researchers indicated that this weak toxicty of the halide tetramethylarsonium salts might be dependent on the halogen type. Thus, we subsequently examined the detailed *in vitro* cytotoxicity of tetramethylarsonium hydroxide (TetMA-OH), which was prepared from synthetic tetramethyarsonium iodide[41] using an anionic ion-exchange resin column, in various murine immmune effector cells, such as splenocytes, thymocytes, Peyer's patch lymphocytes, peritoneal macrophages, alveolar macrophages and bone marrow cells. As a result, we found TetMA-OH had absolutely no cytolethality and/or no pharmacological effects in these immune effector cells *in vitro*.[27] It was suggested that the weak *in vitro* cytotoxicity of tetramethylarsonium iodide might be due to the influence of the iodide ion.[44]

In conclusion, using synthetic pure chemicals, we recently demonstrated that organic arsenic compounds in seafood, such as AsSug,[26] AsCho,[25,33] AsBe[25,28] and TetMA,[27] were not very cytotoxic *in vitro* in murine immune effector cells; we also observed some interesting potent biological effects with AsSug[26] and AsBe.[28] These findings suggest that, at least, the one-time consumption of organic arsenic compounds in seafood does not adversely affect the health of people; however, further examinations will be necessary to clarify the chronic effects, especially the *in vivo* effects.

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